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STRUCTURE OF *Narcissus tazetta* GLUCOMANNAN

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Naturally acetylated glucomannan with MW 31,000 and $[\alpha]_D^{20}$ *-29.1° was isolated from bulbs of* Narcissus tazetta *L. Chemical methods and IR and* ^{13}C *NMR spectroscopies were used to show that the polysaccharide contains 12.9% O-Ac groups and consists of D-mannose and D-glucose in a 1:5.6 ratio that form a linear polymer through* β*-1,4-glycoside bonds.*

Key words: *Narcissus tazetta*, glucomannan, isolation, ^{13}C NMR spectroscopy, structure.

Plants of the *Narcissus* genus are rich in water-soluble polysaccharides (WSPS), most of which are glucomannans [1, 2]. Our goal was to isolate glucomannan from *N. tazetta* bulbs and to study its chemical structure and properties. WSPS were obtained by precipitation with alcohol from the aqueous extract of ground bulbs and were subjected to total acid hydrolysis. Chromatographic analysis (PC and TLC) showed that the hydrolysate contained rhamnose and arabinose in addition to glucose and mannose.

Fractional precipitation by alcohol was used to isolate pure glucomannan from the aqueous solution. Three fractions were obtained in yields of 20% (I), 60% (II), and 8.0% (III). Of these, fraction II contained the highest amount of glucomannan. According to ultracentrifugation, fraction II was homogeneous. Its hydrolysate contained D-glucose and D-mannose in a 1:5.6 ratio. Therefore, fraction II is glucomannan (GM).

GM is a white amorphous powder that dissolves in water and forms viscous solutions that do not produce color with iodine and form a precipitate with solutions of heavy-metal salts.

The molecular weight determined by sedimentation constant is 31,000. The content of O-Ac groups is 12.9%. The IR spectrum has absorption maxima (KBr, ν_{max}) at 1750 and 1240 (ester), 878 (β-glycoside), and 815 cm^{-1} (hexopyranose ring). The IR spectrum of GM is similar to those of mannose-containing polysaccharides (mannans, glucomannans, galactomannans), the chains of which are constructed wholly or from rather extensive 1,4-β-mannopyranose units [3]. It is known that the presence of β-mannoside bonds is responsible for the negative optical activity of solutions of such compounds. GM has a negative rotation angle $[\alpha]_D^{20}$ -29.1° (*c* 1.0, water).

Treatment of GM solutions with Fehling's and NaOH solutions formed regenerated glucomannans that became insoluble in water. However, they had the same monosaccharide composition and identical IR spectra in which ester absorption bands were missing. Therefore, treatment of glucomannan by these solutions partially change it by deacetylation.

The type of bonding and size of oxygenated rings of GM monosaccharide units were determined by periodate oxidation and methylation. Periodate oxidation of GM was complete after 12 days. The consumption of oxidant was 0.94 mole per mole of anhydrous unit. The amount of released formic acid was 0.06 mole. The products of Smith degradation contained mainly erythrite and an insignificant amount of mannose and glycerine. This is consistent with the presence of 1→4 bonds between hexopyranose units and the possible branching or localization of O-Ac groups on C-2 or C-3 in the glucomannan chain.

Hakimori methylation [4] of GM produced the permethylate with $[\alpha]_D^{22}$ -20° (*c* 1.0, $CHCl_3$). Its IR spectrum lacked hydroxyl absorption bands. The GM permethylate was subjected successively to formolysis and hydrolysis. TLC of the products with authentic markers identified 2,3,6-tri-*O*-Me-D-mannose, 2,3,6-tri-*O*-Me-D-glucose, and 2,3,4,6-tetra-*O*-Me-D-mannose in addition to traces of di-*O*-Me-hexose.

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TABLE 1. ^{13}C NMR Spectrum of Depolymerized Glucomannan from *N. tazetta* Bulbs

Monosaccharide fragment of polymer	Chemical shifts, ppm				
	C-1	C-2	C-3	C-4	C-5
β-D-Man*p*	101.3	71.1	72.9	74.1	75.6
β-D-Glc*p*	103.6	74.1	75.6	80.3	78.07

Chemical shift of C-6 is 61.6 ppm.

The negative specific rotation of GM permethylate and the absorption band at 878 cm^{-1} in the IR spectrum are consistent with the presence of a β-glycoside bond. This assumption was confirmed by oxidation of fully acetylated GM by chromium anhydride in glacial acetic acid [5]. Hexoses were not detected in the oxidation products. Thus, hexose units in GM are linked by β-1→4 bonds with a mannose unit on the nonreducible end of the GM.

High-resolution ^{13}C NMR spectra were obtained after partial depolymerization of the GM. The primary structure of the isolated depolymerized GM was unchanged according to the practically identical ratio of monomers and IR spectrum. However, the molecular weight decreased. The comparative characteristics of GM and depolymerized GM of *Narcissus tazetta* bulbs are presented below.

Main parameters	GM	Depolymerized GM
Glc:Man ratio	1:5.6	1:5.7
MW	31000	17000
η_{char}	22.5	10.2

Comparison of ^{13}C NMR spectra of GM with those studied in previous work [6-8] showed a definite similarity of the principal resonances. A comparison of the chemical shifts revealed the assignment of the C signals in the ^{13}C NMR spectrum of GM. Table 1 gives the position of signals in the spetrum (chemical shifts) and their interpretation.

The spectrum of GM contains C signals for β-(1→4) bonds between mannopyranose and glucopyranose units and signals at 21.0-22.84 and 174.0-174.99 ppm corresponding to methyls and acetate carbonyls.

GM was subjected to partial acid hydrolysis to establish the sequence of bonding of monosaccharides in the chain. PC using authentic samples identified in the hydrolysate mannose, glucose, and oligosaccharides [9]: glucosylmannose, mannobiose, mannosylglucose, mannotriose, and mannotetraose.

Results from partial cleavage indicate that GM contains chain portions consisting of fragments with β-glycoside bonds: Man*p*–Glc*p*, Man*p*–Man*p*–Man*p*–Man*p*–. Cellobiose was not detected. Therefore, chain portions with two adjoining glucoses can be eliminated.

In summary, the linear chain polymer can be formulated:

-4-Man*p*(β-1→4)-Man*p*(β-1→4)-Gly*p*(β-1→4)-Man*p*(β-1→4)-Man*p*(β-1→4)→Man*p*(β-1→4)-Man*p*(β-1→4)-Glc*p*(β-1→4)-Man*p*-.

GM of *N. tazetta* bulbs has a structure that is very common for higher plants. However, it differs from previously reported GMs [10, 11] in the ratio of sugars, molecular weight, OAc content, and distribution of hexose units along the linear chain biopolymer.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. Descending chromatography was performed on FN-1.11 paper.

The following solvent systems were used: *n*-butanol:pyridine:water (6:4:3); TLC was performed on silufol plates using systems: MEK:aqueous ammonia (1%) (30:4) and benzene:acetone:water (5:5:1).

Spots were developed using acid aniline phthalate (10-15 min at 105°C) and periodate—$KMnO_4$—benzidine.

GC was carried out on a Chrom-5 instrument with a flame-ionization detector under the following conditions: stainless steel column (200 × 0.3 cm) packed with XE-60 (5%) on chromaton NAW 0.200-0.250 mesh, 200°C, He carrier gas (60 mL/min). Samples of GM were hydrolyzed by H_2SO_4 (1 N) for 5 h at 100°C.

^{13}C NMR spectra were recorded on a Unity 400+ spectrometer (Varian) at 100 MHz working frequency for C using D_2O solutions (3%) of polysaccharides. Methanol was used as an internal standard.

Sedimentation was carried out in a MOM-3170 ultracentrifuge at 50,000 rpm and 20°C for 10 min.

Inactivation of WSPS. Ground air-dried raw material (bulbs, 150 g) was inactivated three times with methanol for 2 h in a 1:10 ratio on a boiling-water bath and filtered. The plants were dried.

Isolation of WSPS. Raw material after inactivation was extracted for 4 h with water in a 1:5 ratio at room temperature. The extraction was repeated twice. The extracts were combined and evaporated to a volume of 0.5 L. Polysaccharides were precipitated by alcohol in a 1:3 ratio. The precipitate was centrifuged off, washed with alcohol, and dried over P_2O_5 in vacuum. Yield 13 g.

WSPS Fractionation. Polysaccharide (3 g) was dissolved in water (400 mL) and treated dropwise with alcohol (75 mL) with vigorous stirring.

The resulting precipitate was centrifuged off and dried. Yield 0.6 g (fraction I). The supernatant was treated again with alcohol (125 mL). The precipitate was worked up analogously. Yield of GM, 1.8 g (fraction II). Fraction III was isolated by adding alcohol (50 mL). Yield 0.24 g.

GM Hydrolysis. GM (0.5 g) was hydrolyzed by H_2SO_4 (3 mL, 1 N) for 16 h on a boiling-water bath, neutralized, and deionized. PC (system 1, developer 1) detected glucose and mannose.

Periodate Oxidation and Smith Degradation of GM. GM (90 mg) was dissolved in water (90 mL), treated with sodium periodate solution (15 mL, 0.25 M), and left in the dark at 20-22°C. Aliquots (1 mL) were taken at certain time intervals. The excess of sodium periodate was titrated with $Na_2S_2O_3$ (0.01 M); formic acid, by NaOH solution (0.01 N). The oxidation product was reduced by $NaBH_4$. The polyalcohol was hydrolyzed by H_2SO_4 (2 mL, 0.5 N) for 8 h at 100°C. PC detected in the hydrolysate glycerine and erythrite (system 1, developer 2) and traces of mannose (system 1, developer 1). Part of the hydrolysate was evaporated to dryness and analyzed by GC.

GM Methylation. GM (0.5 g) was methylated twice using the Hakimori method [5] to produce the fully methylated product. Yield 0.45 g; OCH_3, 42%. The hydrolysate of the permethylate according to TLC (systems 2 and 3, developer 1) contained 2,3,6-tri-*O*-Me-D-mannose, 2,3,6-tri-*O*-Me-D-glucose, and 2,3,4,6-tetra-*O*-Me-D-mannose, and traces of di-*O*-Me-hexose.

GM Acetylation. GM (0.25 g) was dissolved in formamide (40 mL) with stirring, treated with pyridine (40 mL) and acetic anhydride (30 mL), and stirred for five days. The reaction mixture was poured onto ice water. The resulting precipitate was centrifuged off, washed with alcohol, and dried over P_2O_5. Yield 0.20 g.

IR spectrum: hydroxyl absorption band missing, bands at 1240 and 1750 cm^{-1} clearly visible.

Oxidation by Chromium Anhydride. CrO_3 (0.5 g) was dissolved in glacial acetic acid (15 mL), treated with GM acetate (0.2 g), and heated for 4 h at 50°C.

The mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ was dried over anhydrous Na_2SO_4 and evaporated to dryness. The solid was dissolved in H_2SO_4 (1 mL, 1 N) and hydrolyzed for 16 h on a boiling-water bath. The hydrolysate was worked up as usual. PC did not detect hexose.

GM Depolymerization. GM (0.5 g) was dissolved in HCl (50 mL, 0.1 N) and hydrolyzed at 85°C for 45 min.

The hydrolysate was cooled to room temperature and precipitated by ethanol in a 1:3 ratio. The precipitate was separated by centrifugation, washed with alcohol, and dried in vacuum over P_2O_5. Yield of depolymerized GM, 0.22 g.

Partial Hydrolysis of GM. GM (1 g) was dissolved in formic acid (80 mL, 90%), diluted to 45% concentration, and hydrolyzed for 3.5 h at 85°C.

The solution was cooled, centrifuged, evaporated to dryness, and hydrolyzed by H_2SO_4 (0.5 N) for 10 min on a boiling-water bath. The hydrolysate was neutralized and deionized. PC (system 1, developer 1) detected oligosaccharides: glucosylmannose, mannobiose, mannosylglucose, mannotriose, and mannotetraose.

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